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Wing color change by sunlight irradiation in the spotless grass yellow butterfly, *Eurema laeta betheseba* (Lepidoptera, Pieridae)

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Abstract Wing color change of the spotless grass yellow butterfly, *Eurema laeta betheseba*, was compared between specimens exposed to sunlight and others kept in the dark for one month, and between specimens exposed to ultraviolet (wavelength of 254 nm) and green light (532 nm) for 8 h. The results showed that the color of the ventral surface of the hind wings was changed from yellow to yellowish brown by sunlight irradiation. Irradiation of ultraviolet light had more effect on wing coloration than irradiation with green light. The color change in the wings was more marked in males than in females. Wing color did not change in specimens kept in the dark. Microscopic observations showed that the color change was derived from changes in pigments in wing scales. The light reflectance increased in the wavelength region of 400-500 nm, whereas it decreased in the region of 500-650 nm after sunlight irradiation. In the photoluminescence (PL) spectra of the wings measured at room temperature, a broad emission around 500 nm was observed, suggesting the presence of pterin pigments in the scales. The peak intensity of PL spectra became slightly smaller after sunlight irradiation in both sexes. These results indicate that the wing color change in *Eurema laeta betheseba* was due to deterioration of the pterin pigments in the scales as a result of ultraviolet light irradiation.

Key words *Eurema laeta betheseba*, photoluminescence, pterin pigment, reflectance spectrum, seasonal form, wing color.

Introduction

Butterflies have a wide variety of beautiful wing colors, which have attracted scientific and technological interest. Their vividly colorful wings are either due to the selective absorption of light by pigments in the wing scales or to the reflection of light by regularly arranged scales and nanometer-sized structures in the scales (Ghiradella, 1972; Kinoshita and Yoshioka, 2005; Rutowski *et al.*, 2007; Wijnen, 2007). It is known in some species that the coloration of the body and wings of insects is initiated by the stimuli of light, temperature and humidity (Kato and Yamada, 2001; Kato *et al.*, 2010; Yamamoto *et al.*, 2011).

The two main subfamilies, Coliadinae (sulfurs) and Pierinae (whites), of the Pieridae are distinguished from each other by their wing colors; those of coliadine species are yellow to orange, and those of pierine species are white. The wing coloration of pierid butterflies is mainly derived from pigments called pterins consisting in granules or beads studded at the scale crossribs (Yagi, 1954; Rutowski *et al.*, 2005; Edward, 1968; Morehouse *et al.*, 2007). It is also known that the ultraviolet (UV) reflection patterns on the male wings are important for species recognition and sexual identification in the mating behavior of pierid species (Obara and Hidaka, 1968).

The spotless grass yellow, *Eurema laeta betheseba*, is distributed in Honshu, Shikoku and Kyushu in Japan (Kawazoe and Wakabayashi, 1976). This coliadine butterfly is listed in the Red Data List of Japan as one of the most endangered species due to the degradation of habitats with the larval food plant, *Chamaecrista nomame* (Leguminosae) (Ministry of Environment, Japan, 2006). This species has a multivoltine life cycle with several generations per year and passes the winter in the adult stage (Shirôzu, 2006). The adults show two distinct seasonal forms determined by larval photoperiods, the summer and the autumn forms induced by long and short days respectively, and the latter adult form passes the winter (Yata, 1974). The dorsal and ventral surfaces of adult wings are yellow with black markings in both forms.

It is known, however, that the ground color of the ventral surface of the hind wings in the autumn form varies from yellowish brown to reddish brown (Shirôzu, 2006). It has been reported that this color is yellow just after adult emergence but gradually turns yellowish brown with elapsed time in the field (Hasegawa, 1989; Mifune, 2009). However, it is unclear what factors are responsible for this phenomenon.

In this study, effects of sunlight, UV and green light

irradiation on the ground color change of the ventral surface of the hind wings of *E. laeta betheseba* were investigated by spectroscopic measurements and microscopic observations. In addition the difference in wing coloration was compared between male and female adults of *E. laeta betheseba*.

Materials and methods

Insects

Last instar larvae of *E. laeta betheseba* were collected from *C. nomame* at Shirakawa City, Fukushima Prefecture in the Tohoku region of Japan, and reared on leaves of C. nomame under room conditions in Fukushima City late in summer of 2008. Eighteen adults (12 males and 6 females) of the autumn form obtained from the rearing were killed immediately after emergence, and used in two series of laboratory experiments, Experiments I and II, from November to December, 2008.

Experiment I: Effect of sunlight exposure

Male and female adult specimens were prepared with folded wings and the wings cut off from the body at the base with fine scissors. In order to explore the effect of sunlight exposure on the wing color change, the specimens were placed near the window and kept in the dark, respectively for one month in the laboratory of Osaka Prefecture University, Sakai City in Osaka Prefecture. The specimens were placed horizontally on their right side on a desk near the window so that the ventral surface of the left hind wings received sunlight through the window. The duration of sunshine during the experiment (from Nov. 6th to Dec. 6th, 2008) was calculated to be 170 hours from the data of Japan Meteorological Agency, Sakai City, 2008. As a control for the experiment, the other specimens were put into a light-tight box and placed next to the ones which were exposed to sunlight.

Experiment II: Effect of UV light irradiation

In order to explore the effect of UV light irradiation on the wing color change, the ventral surface of the left hind wings cut off from the body of male and female adults was irradiated with UV light of 254 nm using a 15-W low-pressure mercury lamp (a germicidal lamp) with a power density of 1.0 $mWcm^{-2}$ for 8 h at room temperature (around 25°C) in the laboratory of Osaka Institute of Technology. Irradiation of green light (532 nm) was also carried out for the wings of male and female adults using a semiconductor laser with a power density of 1.3 $mWcm^{-2}$ for 8 h at room temperature. The right hind wings cut off from the body were put into a light-tight box.

Photography and spectroscopy for wing surface observation

In both Experiment I and II, the diffuse reflectance spectra of intact wings were measured with a spectrophotometer (Shimazu, UV3600 equipped with an integrating sphere, ISR-3100) with the plane of the wing approximately perpendicular to the probe in the range from 200 to 800 nm. A white reflectance standard ($BaSO_4$) was used as a reference. The ventral surface of the left hind wing cut off from the body was used to analyze the color change of the wings. In order to examine the pigments in the wing scales, the photoluminescence (PL) spectra of the wings were measured using a He-Cd laser (325 nm, 50 mW) as an excitation source. The emitted light from the specimens was detected by a photon-counting system with a photomultimeter through a monochromator.

Microscopy

The morphology and the color of the wings were observed with a laser scanning microscope with a blue laser (Keyence VK9500) and with a scanning electron microscope (SEM, Keyence VE8800) at an acceleration voltage of 8 kV. For the SEM observations, wing specimens were gold coated by sputtering for 30 s (Sanyu Electron SC-701). The sputtered specimens were attached to the sample holder using carbon double-sided tape. The coloration of the wings was also observed by the naked eye.

Results

Experiment I

The ground color of the ventral surface of the left hind wing gradually changed from yellow to yellowish brown in male specimens subjected to one-month exposure to sunlight, whereas that of both hind wings remained yellow in male specimens kept in the dark for one month (Fig. 1). The color change of the wings as a result of sunlight irradiation stopped within one month. Comparing specimens of both sexes exposed to sunlight, the resultant ground color of the ventral hind wing surface was darker in males than in females (Fig. 2). Microscopic observations showed that the change in wing color as a result of sunlight exposure was derived from that in scale color, but there was no difference in the morphology of scales (Fig. 3). The density of the scales of the males was slightly higher than that of the females.

In the diffuse reflectance spectra from the ventral hind-wing surface, the reflectance increased in the wavelength region of 400-500 nm and decreased in the region of 500-650 nm after the one-month sunlight irradiation for both sexes (Fig. 4). The sunlight irradiation did not affect the reflectance of the ventral hind wing surface in visible

wavelengths longer than 650 nm (about 65% for both sexes) and in the ultraviolet range shorter than 400 nm (about 5% in female and a few percent in male).

The SEM observations showed that the scales on the ventral surface of the hind wing consist of thick longitudinal ribs at intervals of approximately 1.7-1.8 μm with a series of smaller cross-ribs separated by 0.9-1.1 μm (Fig. 5). The scales were densely packed with beads: numerous oval-shaped beads were seen hanging down from the longitudinal ribs and cross-ribs into the scale interior. The density of beads was higher in males than in females.

Experiment II

The irradiation of 254 nm light had a similar effect on the reflectance of the hind wing surface: it increased in the wavelength region of 400-500 nm and decreased in the region of 500-650 nm after the irradiation when compared with that of a hind wing kept in the dark (Fig. 6). On the other hand, the reflectance of the hind wing after the irradiation at 532 nm was almost the same as that of the hind wing kept in the dark.

A broad emission peaking at around 500 nm with a shoulder around 450 nm was observed in all the photoluminescence spectra of the ventral surfaces of hind wings for both sexes (Fig. 7). For both sexes, the intensity of emission was slightly smaller in hind wings exposed to sunlight than in those kept in the dark. Another specimen showed almost the same photoluminescence spectra as shown in Fig. 7.

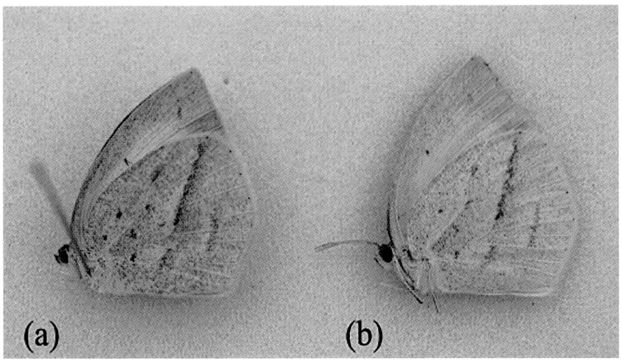


Fig. 1. The ventral surface of the left wings of male *E. laeta betheseba* adults exposed to sunlight (a) and kept in the dark (b) for one month.

Discussion

In this study the ventral surface of hind wings in the autumn form of *E. laeta betheseba* gradually changed from yellow to yellowish brown when the wings were exposed to sunlight. It was also proved in this study that irradiation by UV light (254 nm in wavelength) has an effect similar to that of sunlight. Thus the results of this study demonstrate that a primary factor responsible for the color change of ventral hind wings in the autumn form adults after emergence (Hasegawa, 1989; Mifune, 2009) is UV light

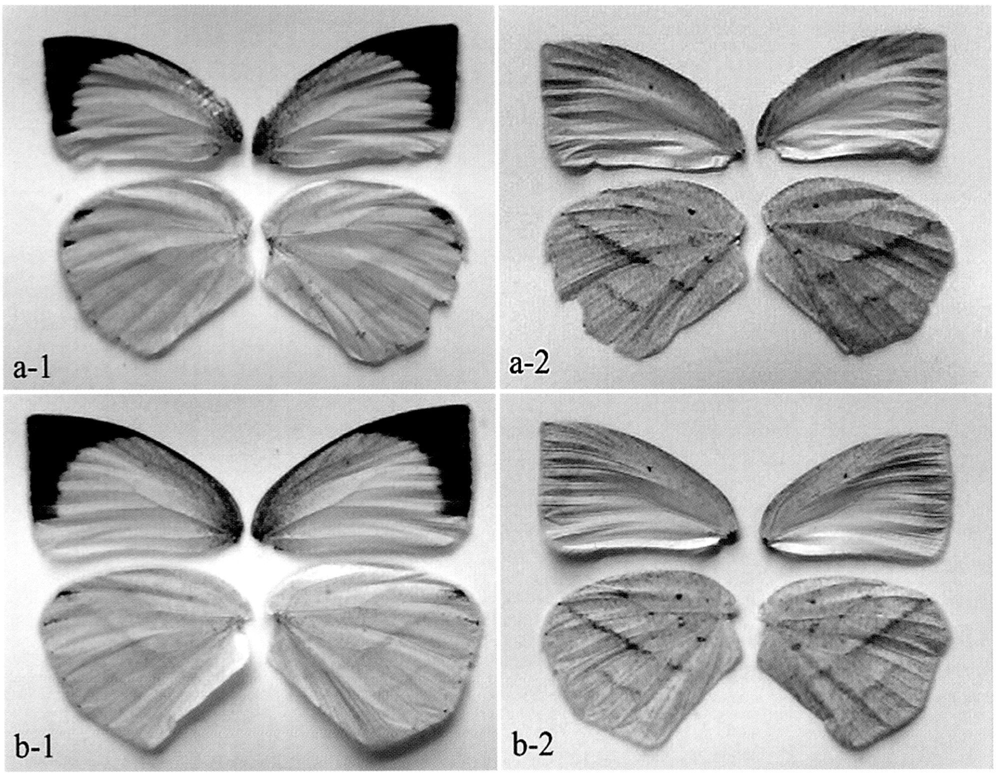


Fig. 2. The dorsal (left) and ventral (right) surfaces of the wings of male (top) and female (bottom) *E. laeta betheseba* adults placed horizontally on their right side on a desk near the window so that the ventral surface of the left hind wing was exposed to sunlight for one month. a-1; dorsal surface of male wings, a-2; ventral surface of male wings, b-1; dorsal surface of female wings, b-2; ventral surface of female wings.

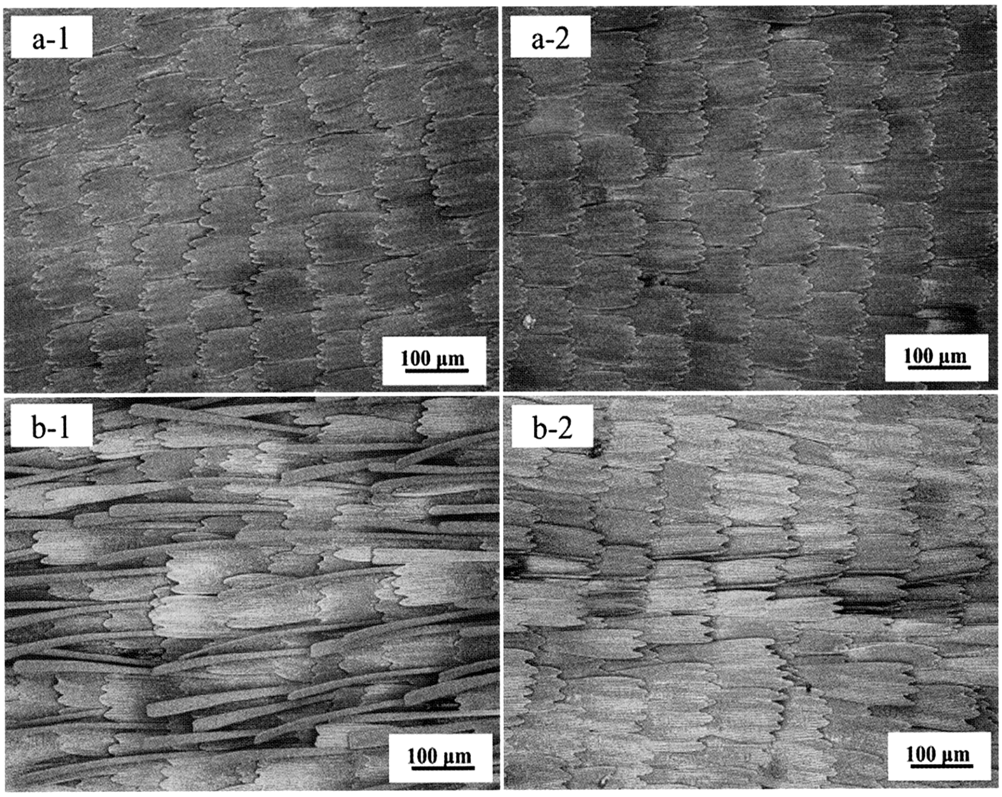


Fig. 3. Microscopic views of the ventral surface of the right and left hind wings of male (top) and female (bottom) *E. laeta betheseba* adults. The same specimens as shown in Fig. 2 were used. a-1; male right hind wing without sunlight irradiation, a-2; male left hind wing with sunlight irradiation, b-1; female right hind wing without sunlight irradiation, b-2; female left hind wing with sunlight irradiation.

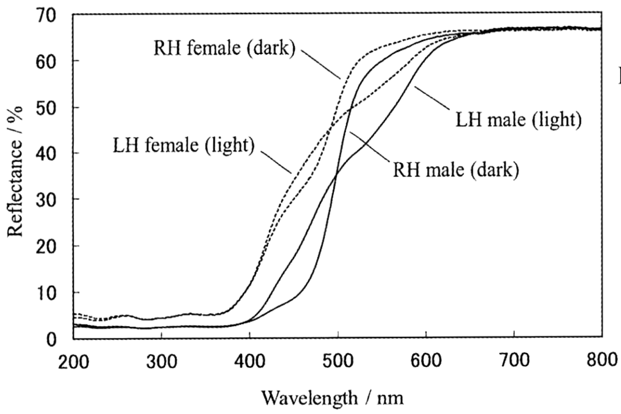
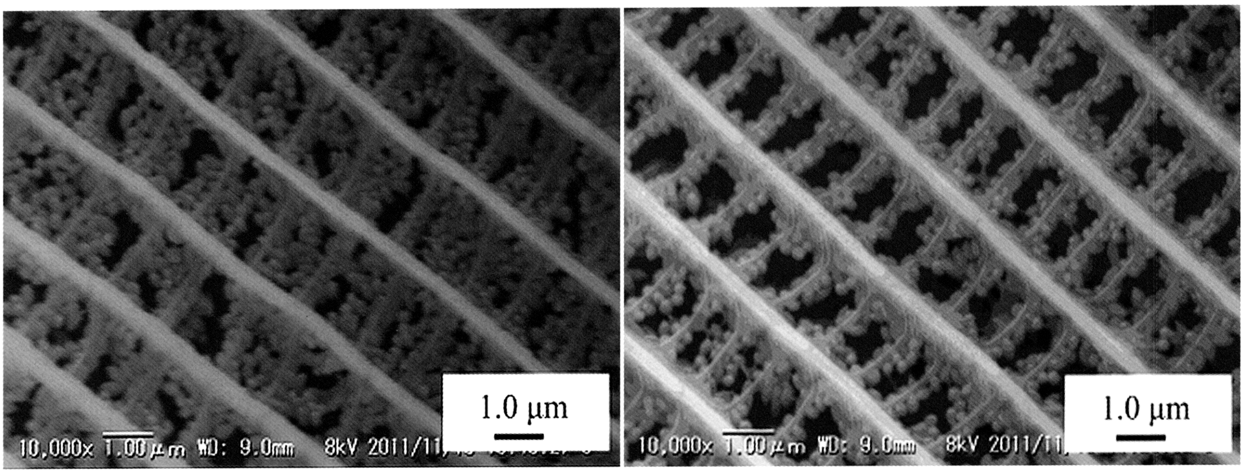


Fig. 4. Reflectance spectra of the ventral surface of the hind wings of male and female *E. laeta betheseba* adults. The same specimens as shown in Fig. 2 were used. RH and LH stand for right and left ventral hind wings, respectively. "light" and "dark" stand for sunlight irradiation and without sunlight irradiation, respectively.



(a) (b)

Fig. 5. SEM photographs of a scale on the ventral hind wing of male (a) and female (b) *E. laeta betheseba* adults.

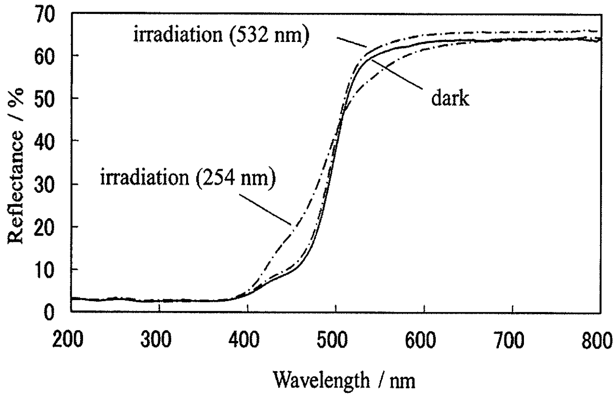


Fig. 6. Reflectance spectra of the ventral surface of the hind wings of male *E. laeta betheseba* adults exposed to irradiation of 254 and 532 nm light for 8 h and kept in the dark at room temperature for more than one month.

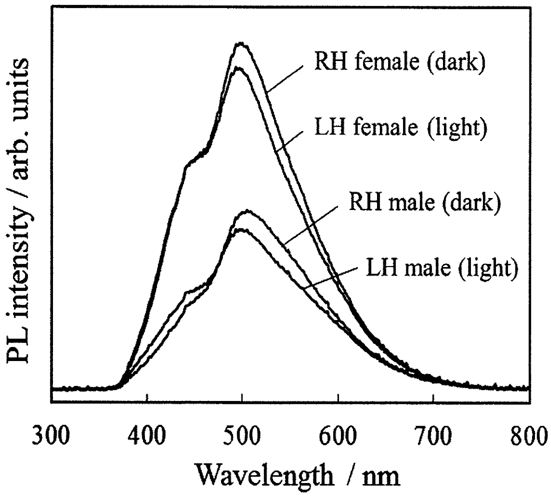


Fig. 7. Photoluminescence spectra of the ventral surface of the hind wings of male and female of *E. laeta betheseba* adults. The same specimens as shown in Fig. 2 were used. RH and LH stand for right and left hind wings, respectively. "light" and "dark" stand for sunlight irradiation and without sunlight irradiation, respectively.

in sunlight. The results are consistent with the wide color variation of ventral hind wings of the autumn form adults found in the field. It should be noted that the color change of ventral hind wings by sunlight occurs even in dead and dried specimens in this species. Jones *et al.* (1985) clarified by rearing experiments that the Australian subspecies, *E. laeta lineata*, has two seasonal forms similar to those of the Japanese subspecies, *E. laeta betheseba*. However, Jones *et al.* (1985) and Jones (1992) failed to find factors causing the ground color of ventral hind wings to change from yellow to brown in the autumn form of *E. laeta lineata* by controlling temperature, photoperiod and humidity. It is inferred from the results of this study that the browning of ventral hind wings in the Australian subspecies is induced by UV light in sunlight.

The measurement of diffuse reflectance spectra in this study showed that the color change of ventral hind wings in *E. laeta betheseba* occurred at a wavelength between 400 and 650 nm: it increased at 400-500 nm and decreased at 500-650 nm after the one-month sunlight irradiation. The results also demonstrate that the color change of the ventral surface of the hind wing by sunlight is caused by UV light. In pierid butterflies wing reflectance at the wavelength of 400-500 nm correlates with the yellowish color (Stavenga and Leertouwer, 2007) derived from pterins in scales (Yagi, 1954; Rutowski *et al.*, 2005). From the reflectance spectra, light in wavelengths of less than 400 nm is absorbed in the wings. The absorption peak of zanthopterin was reported to be about 390 nm (Wijnen *et al.*, 2007). Therefore, light less than 400 nm in wavelength is effective in creating color change on the ventral surface of the hind wings. Photoluminescence spectra of the ventral surface of hind wings measured in this study showed a broad emission peaking at around 500 nm with a shoulder around 450 nm. The spectra are considered similar to those of already reported pterin pigments (Parker *et al.*, 1979). Since the intensity of emission slightly decreased after sunlight irradiation, it seems that the color change of ventral hind wings is due to the deterioration of pterin pigments in the scales in *E. laeta betheseba*.

The SEM observation revealed that each scale has numerous oval-shaped beads hanging down from longitudinal ribs and cross-ribs into the scale interior in this species. It is possible that these beads consist of pterin pigments as in other pierids (Yagi, 1954; Rutowski *et al.*, 2005). The ground color of the ventral hind wings became darker in males than in females after sunlight irradiation in this species, which may be related to the finding that the bead density was higher in males than in females.

Tanaka *et al.* (2005) inferred from field observations that this species has two peaks of adult emergence in autumn with individuals emerging later having brownish ventral hind wings. Moreover, the ground color of the ventral hind wings in the autumn form adults collected in the Yaeyama Islands, southwestern Japan (Kawazoe and Wakabayashi, 1976) and Shimane Prefecture, western Japan (Mishima, 1989) was sometimes extremely dark brown, and these were therefore considered to be a different subspecies (Kawazoe and Wakabayashi, 1976; Inomata, 2005). It is also inferred from the results of our study that these individuals had been exposed to UV light in sunlight intensely and/or for a long time after emergence.

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摘　要

太陽光照射によるツマグロキチョウの翅の色彩変化
(棚橋一郎・御船藤志・平井規央・石井　実)

ツマグロキチョウ*Eurema laeta betheseba*の腹側翅に太陽光，254 nmおよび532 nmの波長の光を室温で照射したときの翅の色彩変化について検討した．雌雄のツマグロキチョウの翅の反射スペクトルおよびフォトルミネッセンススペクトルを測定し，雌雄での翅の色彩変化の違いについても検討した．腹側翅は，太陽光と254 nmの光照射により次第に黄色から黄褐色に色彩が変化した．一方，暗所に保存した翅や532 nmの光を照射した翅には色彩の変化が見られなかった．翅の反射スペクトルにおいて，太陽光照射後の反射率は，照射前と比べて，400-500 nmの波長領域で大きくなり，500-650 nmで小さくなることが分かった．このようなスペクトルの変化は雄の翅の方が雌の翅よりも大きかった．色彩が変化する前後の翅をレーザー顕微鏡により観察したところ，翅の色彩変化は鱗粉の色彩変化に起因していることが分かった．ツマグロキチョウの翅の黄色から黄褐色は，鱗粉に存在している顆粒（ビーズ）中のプテリン系色素によると考えられる．雄の鱗粉には雌の鱗粉に比べ，ビーズが高密度で存在し，このことは，雄の翅の色彩変化が雌よりも大きい要因であると考えられる．室温における腹側翅のフォトルミネッセンススペクトルからプテリン系色素の存在が分かり，太陽光に含まれる紫外線によりプテリン系色素が変質し翅の色彩変化が起こるものと考えられる．

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